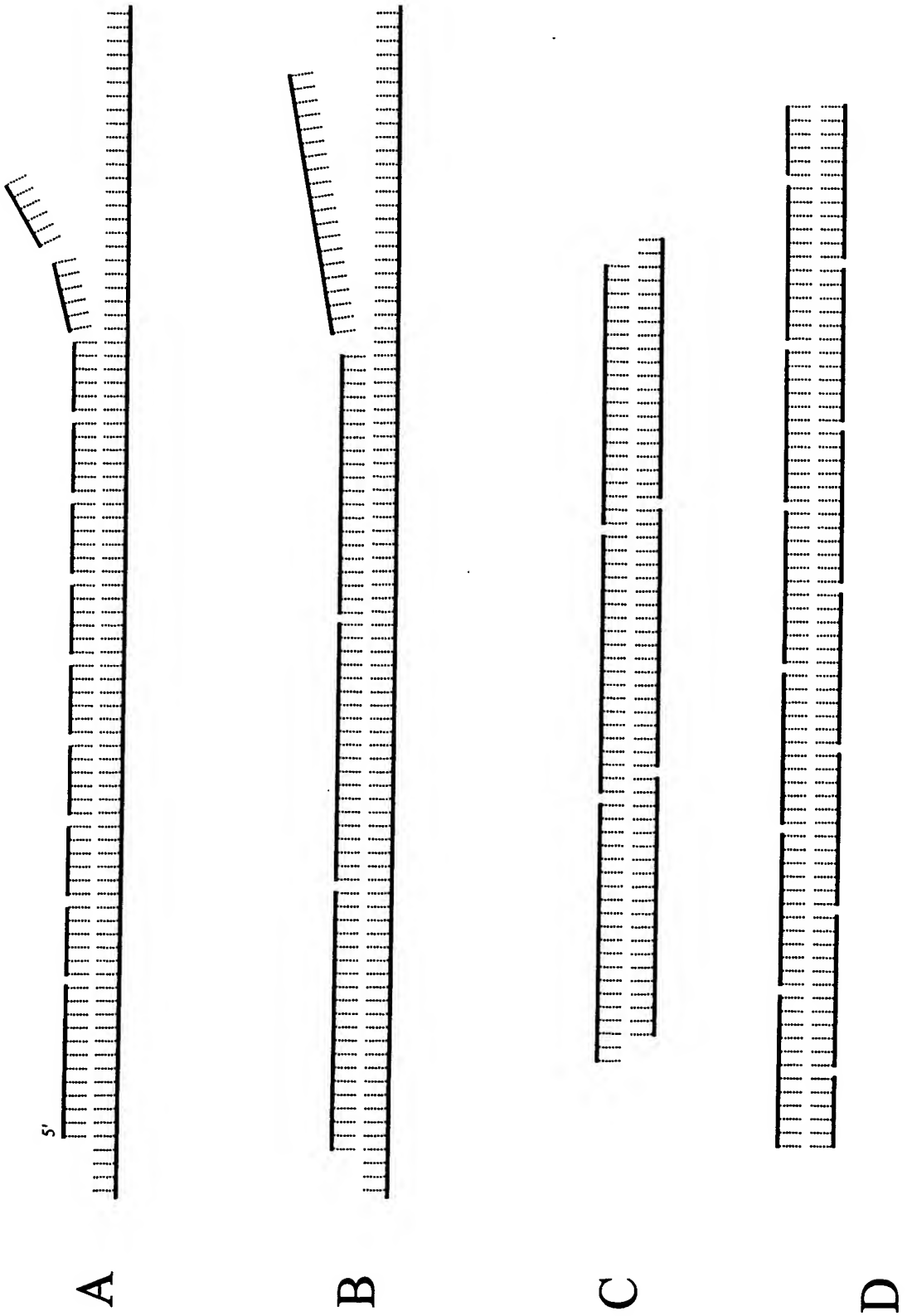

FIG. 1
(PRIOR ART)

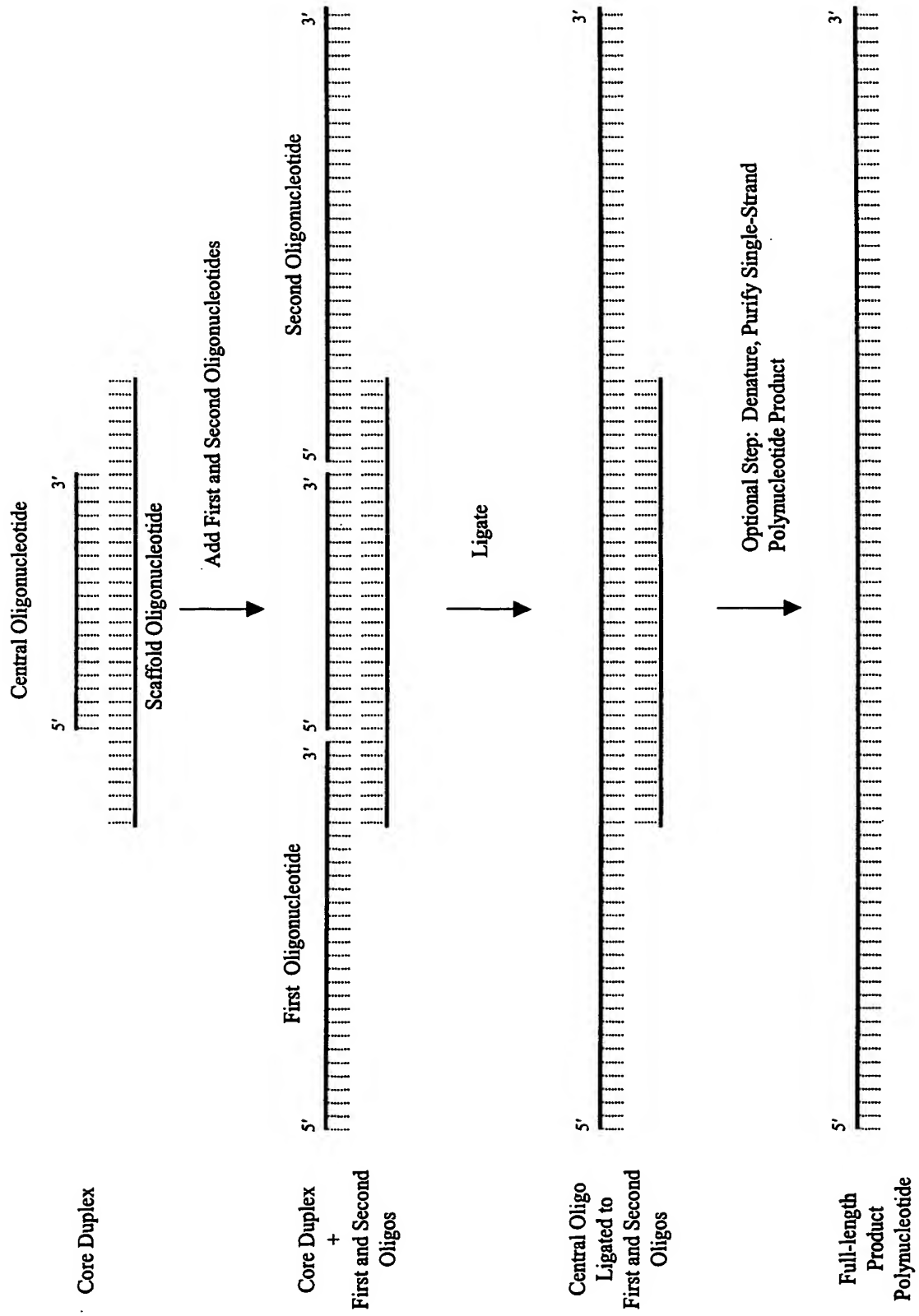
Central Oligonucleotide
5'
3'
Scaffold Oligonucleotide
Add First and Second Oligonucleotides
Second Oligonucleotide
3'
5'
3'
5'
First Oligonucleotide
5'
3'
5'
Ligate
3'
5'
Optional Step: Denature, Purify Single-Strand
Polynucleotide Product
5'
3'
Core Duplex
Core Duplex
+
First and Second
Oligos
Central Oligo
Ligated to
First and Second
Oligos
Full-length
Product
Polynucleotide

FIG. 2A

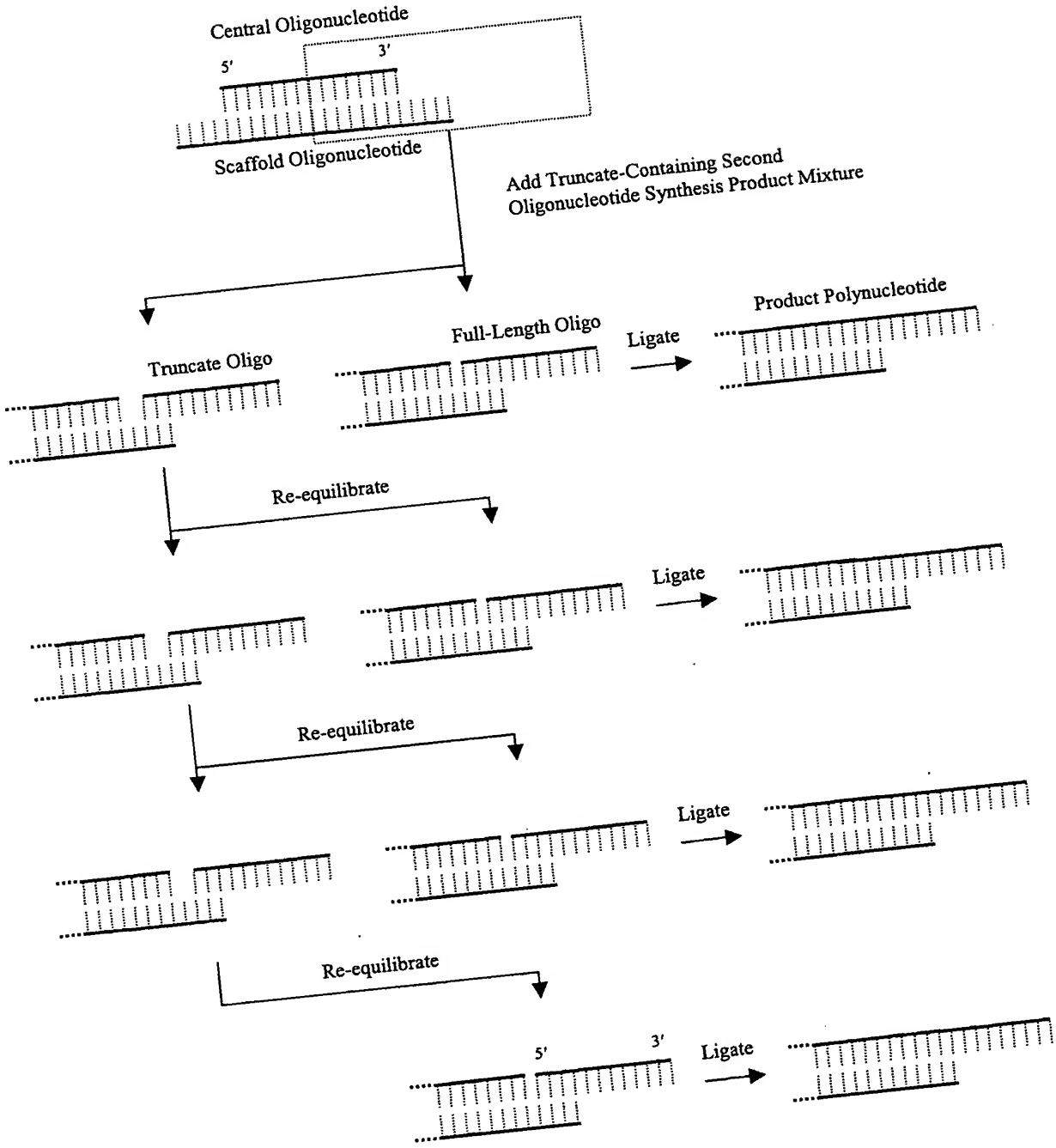
Central Oligonucleotide
5'
3'
Scaffold Oligonucleotide
Add Truncate-Containing Second Oligonucleotide Synthesis Product Mixture
Truncate Oligo
Full-Length Oligo
Ligate
Product Polynucleotide
Re-equilibrate
Ligate
Re-equilibrate
Ligate
Re-equilibrate
5'
3'
Ligate

FIG. 2B

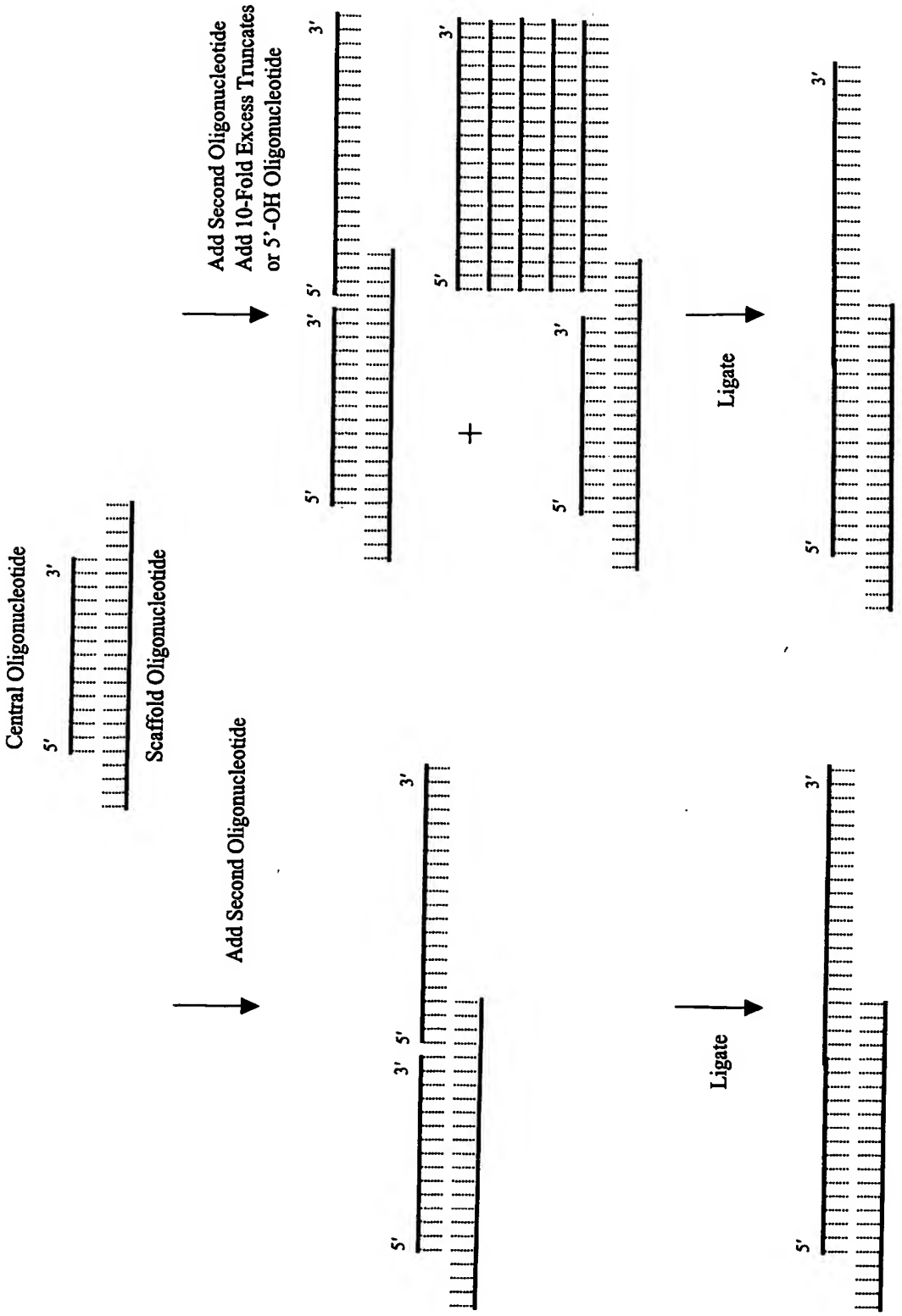
Central Oligonucleotide
Scaffold Oligonucleotide
Add Second Oligonucleotide
Add Second Oligonucleotide
Add 10-Fold Excess Truncates
or 5'-OH Oligonucleotide
Ligate
Ligate
5'
3'

FIG. 3

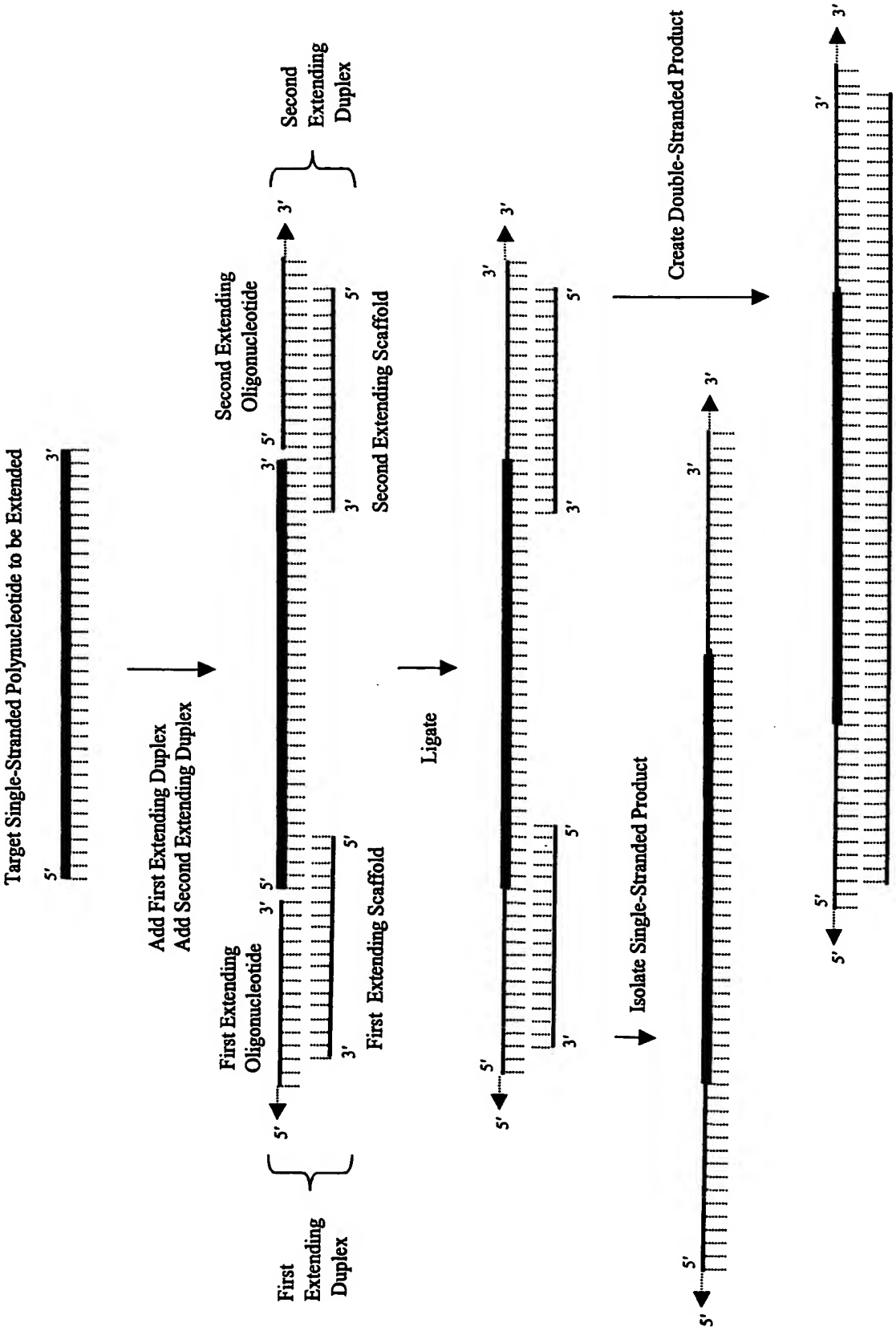
Target Single-Stranded Polynucleotide to be Extended
Add First Extending Duplex
Add Second Extending Duplex
First Extending Oligonucleotide
First Extending Scaffold
First Extending Duplex
Second Extending Oligonucleotide
Second Extending Scaffold
Second Extending Duplex
Ligate
Isolate Single-Stranded Product
Create Double-Stranded Product

FIG. 4A

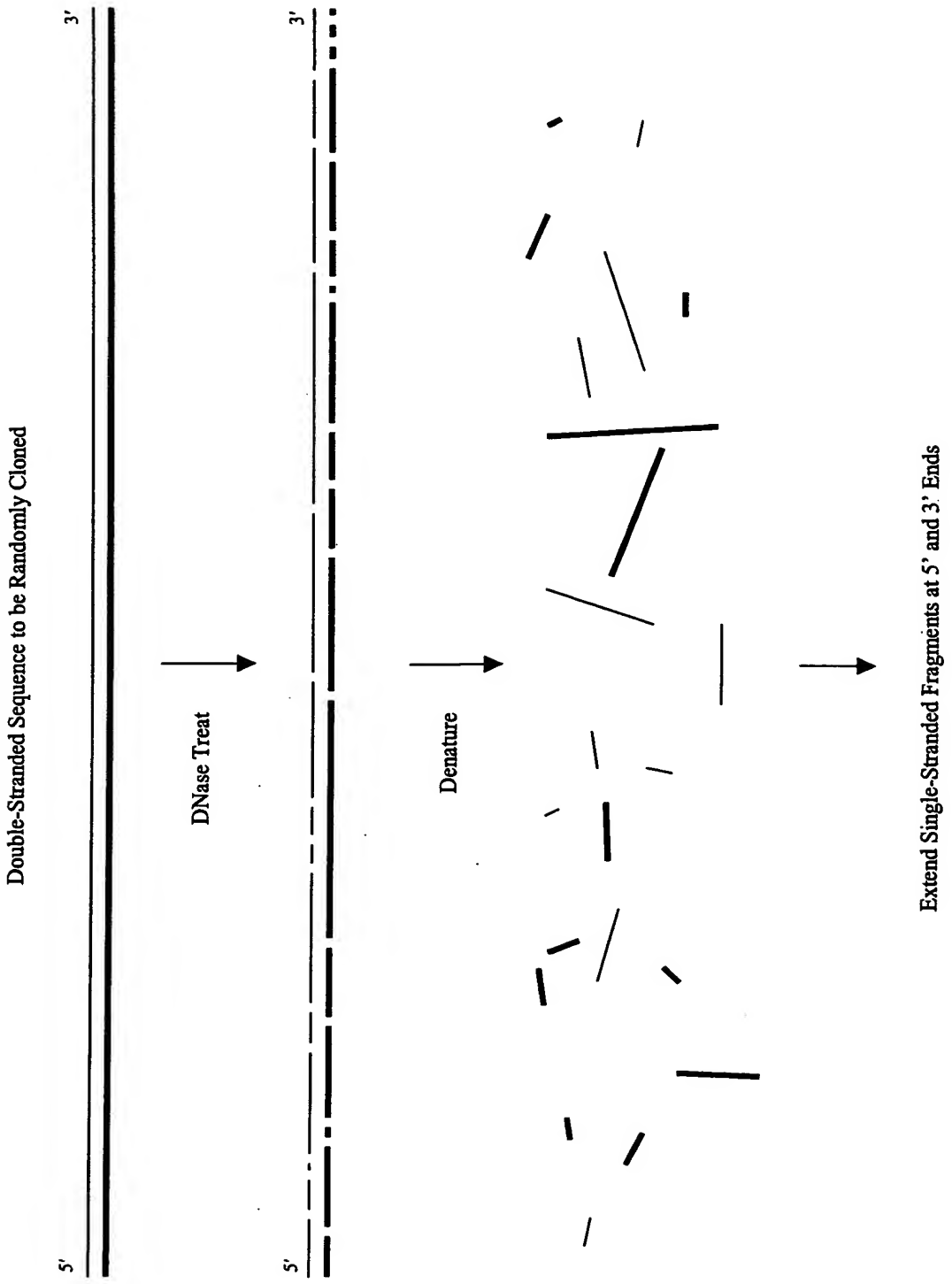
Double-Stranded Sequence to be Randomly Cloned
3'
5'
DNase Treat
3'
5'
Denature
Extend Single-Stranded Fragments at 5' and 3' Ends

FIG. 4B

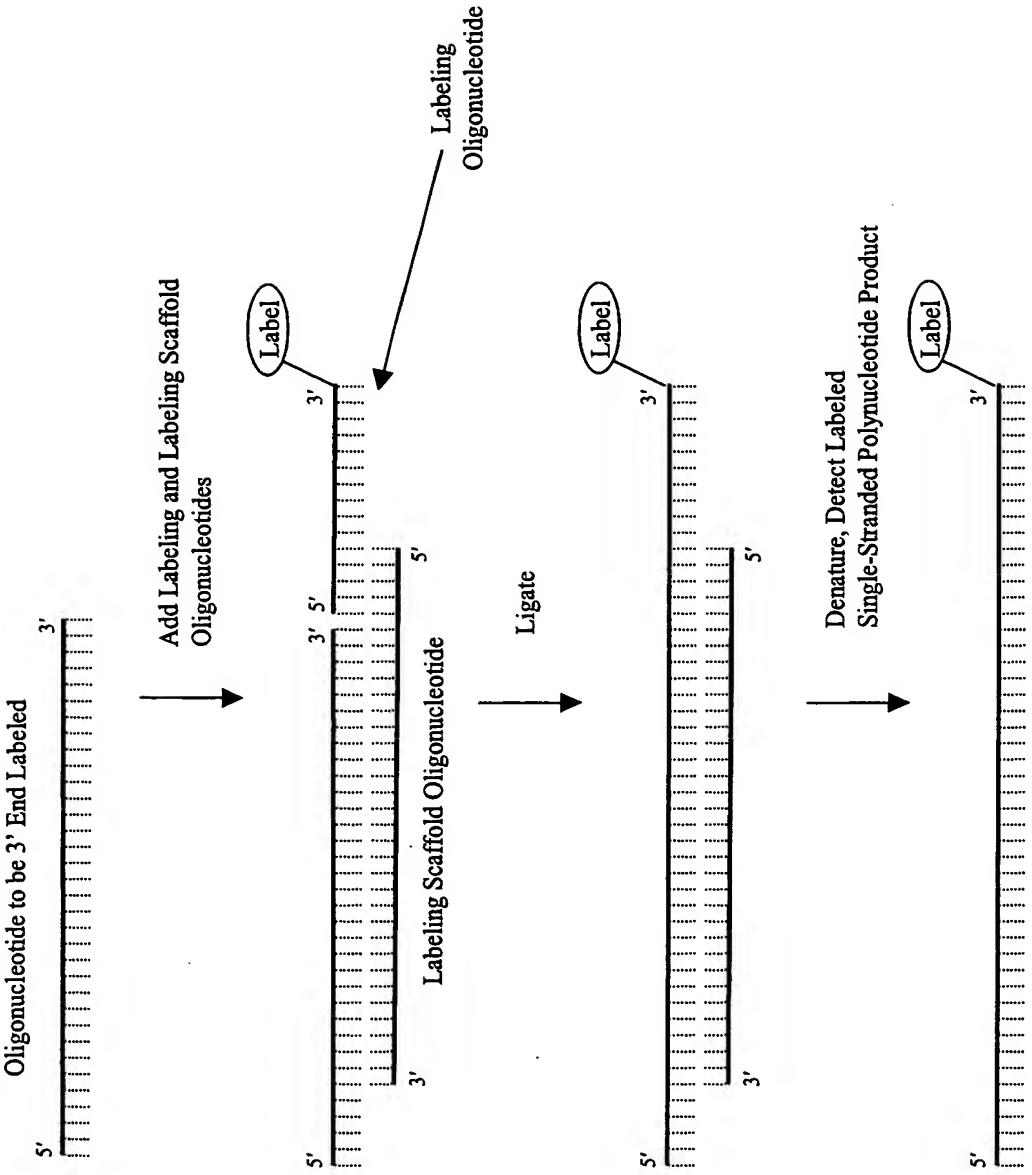
Oligonucleotide to be 3' End Labeled
5'
3'
Add Labeling and Labeling Scaffold Oligonucleotides
Label
Labeling Oligonucleotide
Labeling Scaffold Oligonucleotide
Ligate
Denature, Detect Labeled Single-Stranded Polynucleotide Product

FIG. 5A

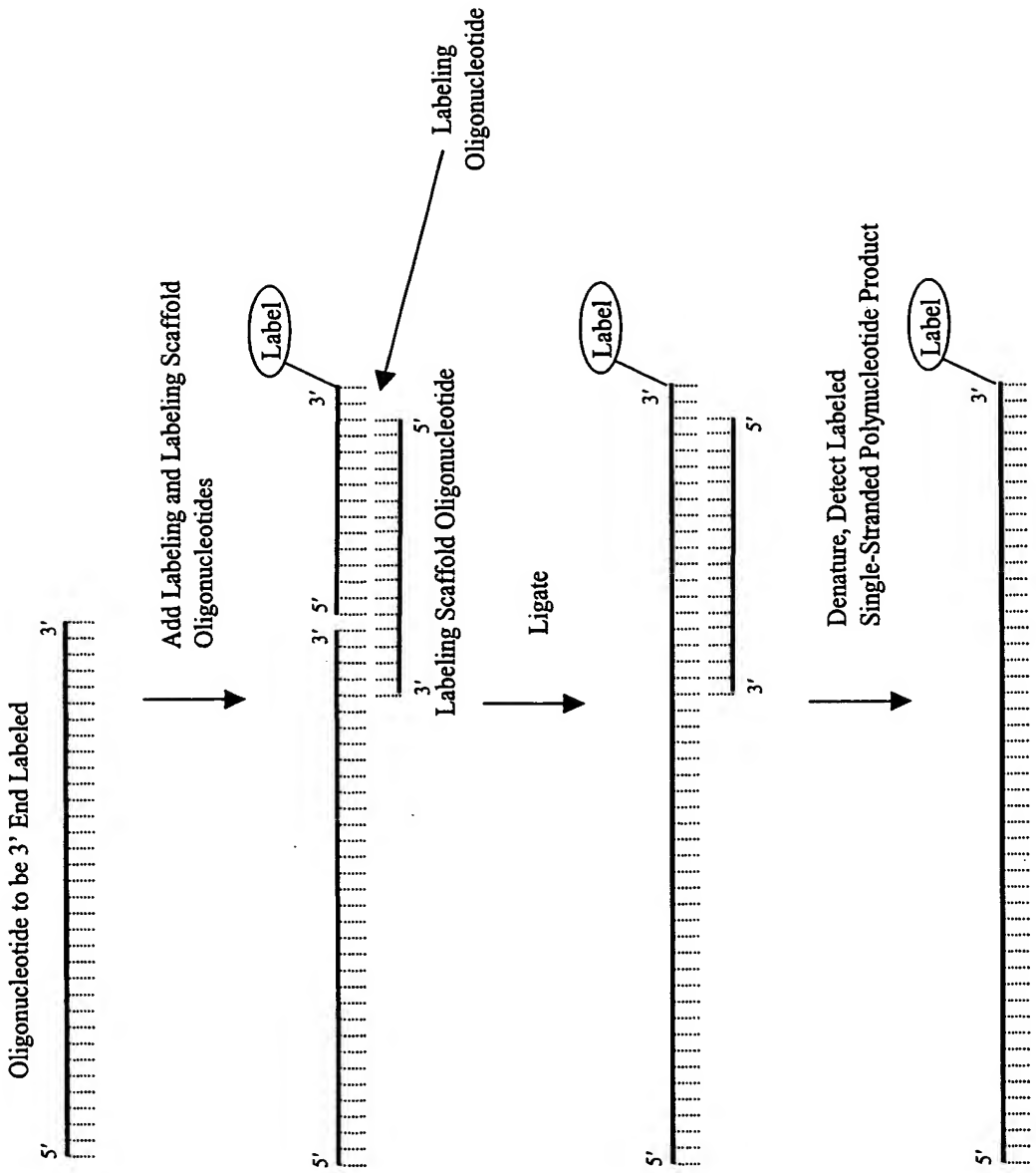
Oligonucleotide to be 3' End Labeled
3'
5'
Add Labeling and Labeling Scaffold Oligonucleotides
Label
Labeling Oligonucleotide
3'
5'
5'
3'
3'
5'
Labeling Scaffold Oligonucleotide
Ligate
Label
3'
5'
5'
3'
Denature, Detect Labeled Single-Stranded Polynucleotide Product
Label
3'
5'

FIG. 5B

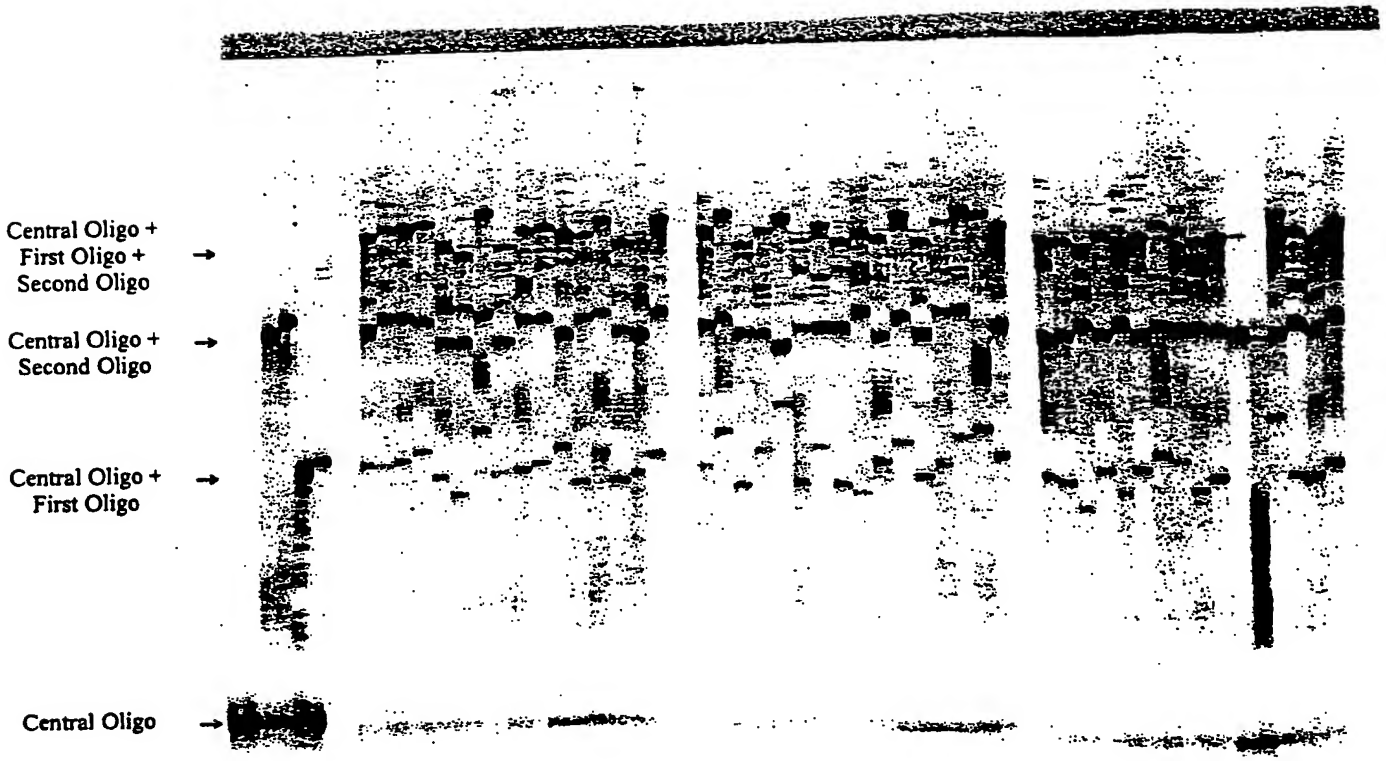
Central Oligo + First Oligo + Second Oligo
Central Oligo + Second Oligo
Central Oligo + First Oligo
Central Oligo

FIG. 6

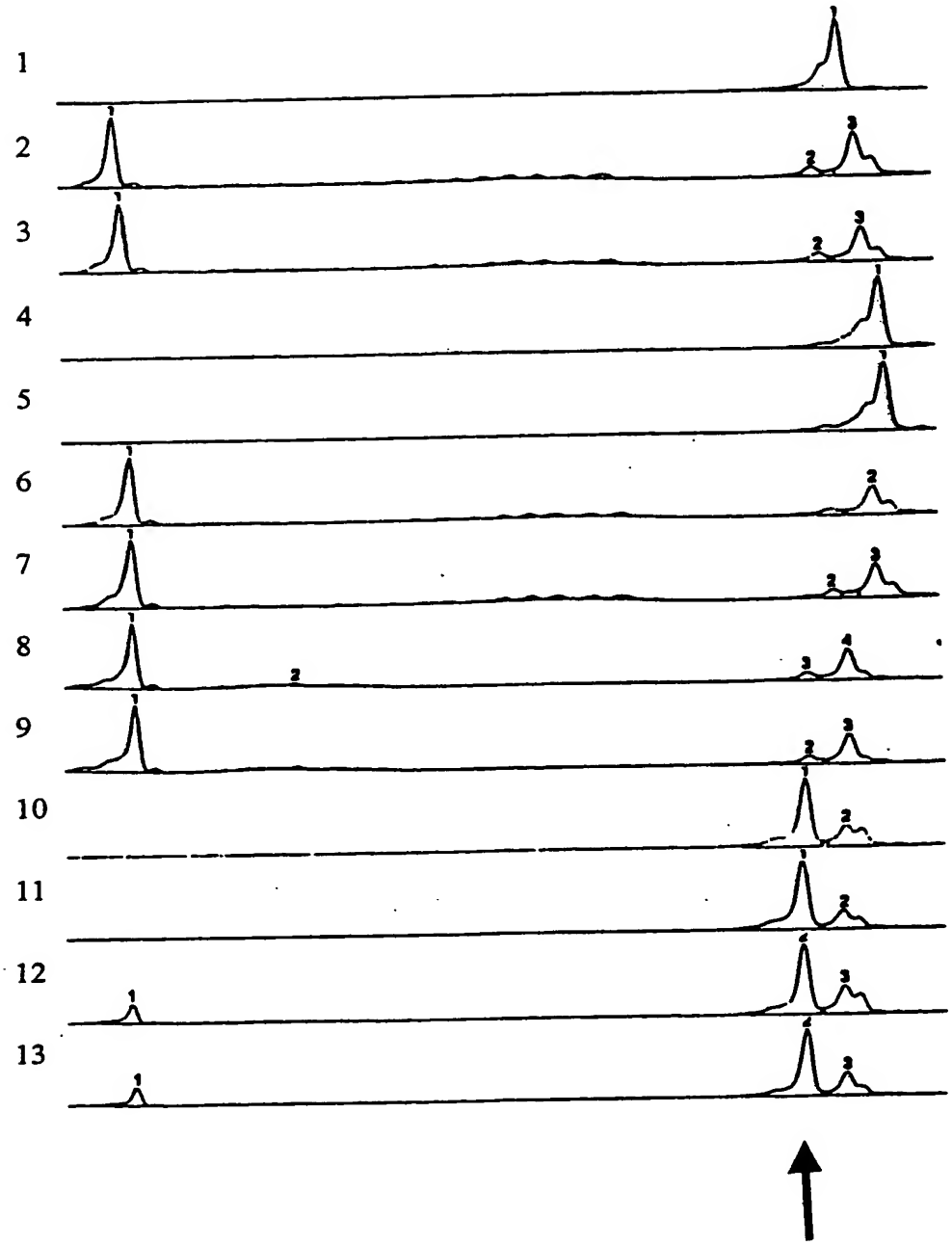

FIG. 7

FIG. 8

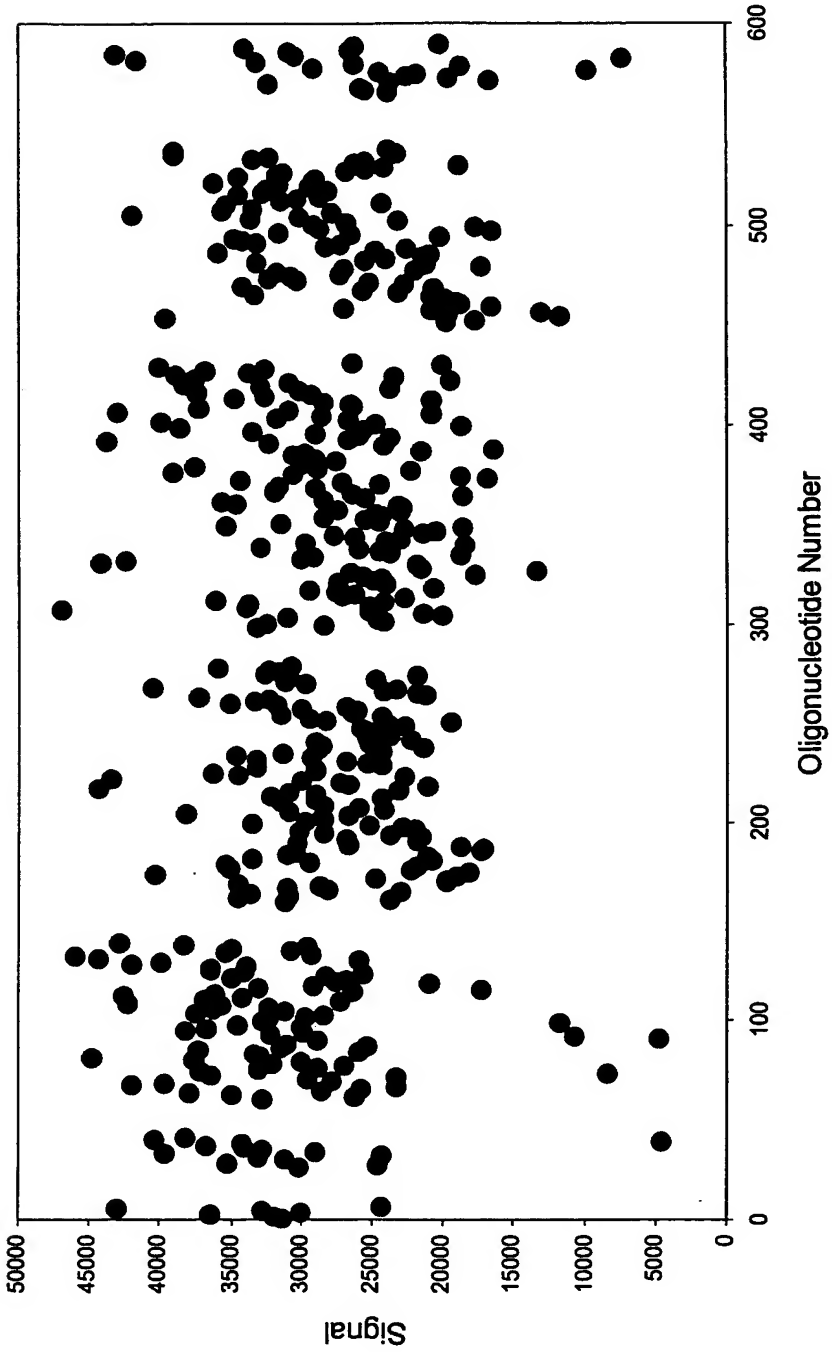
Signal
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
Oligonucleotide Number
0
100
200
300
400
500
600

FIG. 9

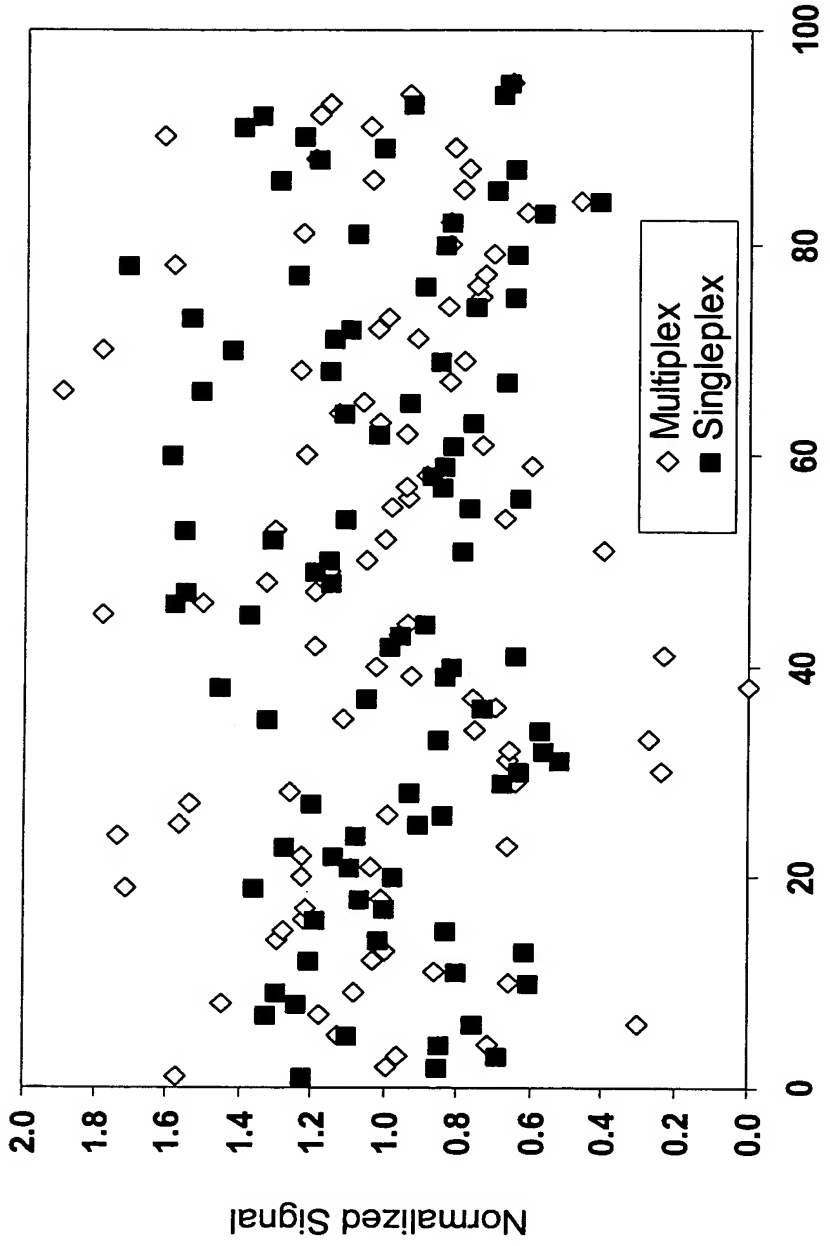
Normalized Signal
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
Oligonucleotide Number
Multiplex
Singleplex

FIG. 10